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# Interferon-induced STAT 1 activation in malignant melanoma cells and tumor tissues

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**Background:** STAT 1, a member of signal transducers and transcription activators of STAT family proteins, has been implicated as important mediator of interferon (IFN) signaling. Functional activation of STAT 1 requires tyrosine and serine phosphorylation. Defects in its expression or activation in response to IFNs were observed in numerous pathological conditions including cancer.

**Purpose:** To explore cancer-associated impaired STAT 1 response to IFNs that may in clinical situation affect sensitivity of malignant melanoma to immunotherapy.

**Methods:** The inducibility of serine (S 727) and tyrosine (Y 701) phosphorylation by IFN- $\alpha/\gamma$  was assessed in 21 melanoma cell lines and in 35 primary cultures derived from melanoma patients. STAT 1 levels and inducibility of its activated phosphoforms were detected by Western blot analysis using specific polyclonal and monoclonal antibodies.

**Results:** All cell lines as well as patient melanoma samples expressed STAT 1 with variable signal intensity. Significant impaired IFN-induced STAT 1 S 727 phosphorylation was observed in both model systems with average of 77% of non-responders recorded in patient melanoma cells and 76% in melanoma cell lines. Failure of PY 701 induction occurred in patient samples (63% after IFN- $\alpha$  and 34% after IFN- $\gamma$  induction) and in a lesser degree in cell lines (i.e. response absence to IFN- $\alpha$  in 5 and to IFN- $\gamma$  in 2 melanoma lines). On the basis of detail analyses of patient melanoma cells with respect to the inducibility of STAT 1 phosphorylation by IFNs, four categories of patients could be distinguished: a) activation on both S 727 and Y 701, b) not inducible response, c) activation on Y 701 but not on S 727, d) heterogeneous response.

**Conclusions:** The study clearly demonstrates STAT 1 functional abnormalities in melanoma cells. Clinical study is now in progress to establish the significance of *in vitro* STAT 1 activation for predicting the patients response to IFN-based therapy and to explore biological consequences in cases responding *in vitro* to IFN-induced STAT 1 activation on only one of the critical amino acid residues.

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# Multicenter phase III randomized trial of polychemotherapy (CVD regimen) versus the same chemotherapy (CT) plus subcutaneous (SC) interleukin-2 (IL-2) and interferon-alpha-2b (IFN) in metastatic melanoma.

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**Background:** Systemic medical management has failed to significantly improve the survival of patients with metastatic melanoma. The results of the currently available therapies are controversial and the combined biochemotherapy seems to be interesting. We performed a phase III study using out-patient concurrent biochemotherapy (BC) versus CT as first-line approach in order to compare the two treatments in terms of overall survival (OS), other than response rate (RR), time to progression (TTP), and toxicity.

**Material and Methods:** From 02/99 to 12/02, 147 pts were enrolled (8 of whom ineligible) with the following characteristics: stage IV non-choroidal melanoma; ECOG performance status (PS) 0-2; absence of brain metastases. Pts were stratified according to site of metastases (visceral vs soft tissues) and prior adjuvant IFN therapy. CVD consisted of Cisplatin 30 mg/m<sup>2</sup> (days 1-3), Vinorelbine 2.5 mg/m<sup>2</sup> (only day 1), DTIC 250 mg/m<sup>2</sup> (days 1-3), where BC consisted of the same CT plus IL-2 9 MIU sc (days 1-5, 8-12) and IFN- $\alpha$  2b 5 MU/m<sup>2</sup> sc (days 1-5). The whole treatment was repeated every 21 days and response was assessed every 2 cycles. 69 and 70 pts receiving CVD (arm A) and BC (arm B), respectively. The Pts characteristics were: median age 50 yrs old (range 19-70), sex (89 males

and 50 females) and PS (0 in 136 pts, 2 in one pt and missing for 2 pts). Then, the visceral/nonvisceral site ratio was 100/39. Forty pts were previously treated with adjuvant IFN- $\alpha$ .

**Results:** By intent to treat analysis the RR was 21% (95% CI: 13-31%) for arm A vs 27% (95% CI: 18-38%) for arm B, with 3% of complete responses only in BC arm. The median TTP for arm A was 6 vs 7 mos for arm B (p=0.7706) and the median OS was 12 mos for arm A vs 11 mos for arm B (p=0.7519). The main side effects observed in BC arm were 36% of G3 fever and 20% of G3-4 asthenia.

**Conclusions:** Complexively, the toxicity was manageable in out patient setting and the feared vascular leak syndrome associated with iv high dose IL-2, with the consequent pulmonary edema and kidney failure, was not observed.

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# Randomised trial of dacarbazine versus BOLD chemotherapy combined with natural or recombinant alpha-interferon in patients with advanced melanoma

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**Background:** Polychemotherapy regimens have yielded higher response rates than the standard agent dacarbazine (DTIC) in advanced melanoma in some studies.  $\alpha$ -interferon (IFN) may act synergistically with chemotherapy. This randomised phase II study compared the efficacy of DTIC and BOLD chemotherapy combined with either natural  $\alpha$  IFN (Finnferon- $\alpha$ ® or recombinant  $\alpha$  IFN-2b (Introna®) in patients with advanced melanoma.

**Patients and methods:** 108 patients were enrolled, of whom 106 were eligible and formed the basis for the efficacy analyses. 56% of the patients had abdominal visceral and/or bone involvement. Patients were randomised to receive A) DTIC plus Finnferon- $\alpha$ ® (n=25), B) BOLD plus Finnferon- $\alpha$ ® (n=31), C) DTIC plus Introna® (n=25) or D) BOLD plus Introna® (n=25). The dose of DTIC was 250 mg/m<sup>2</sup> i.v. days 1-5. The BOLD regimen contained DTIC 200 mg/m<sup>2</sup> i.v. days 1-5, vincristine 1 mg/m<sup>2</sup> i.v. days 1 and 4, bleomycin 15 mg i.v. days 2 and 5 and CCNU 80 mg orally day 1. The dose of IFN was 3x10<sup>6</sup> IU s.c. daily starting on day 8 for 6 weeks and 6x10<sup>6</sup> IU three times weekly thereafter.

**Results:** Overall response rates were: arm A: 8%, arm B: 13%, arm C: 12% and arm D: 24%. The differences were not statistically significant. However, there was a trend in favour of BOLD plus recombinant  $\alpha$  IFN-2b. The BOLD arms produced 6 out of 8 complete responses (CR). All CRs occurred in the patients with metastases limited to the soft tissue and/or lung. The median duration of CR was 46.1 months. The median survival was 9.0 months (range 0.5-84.6+) and the median time to progression was 3.2 months (range 0.2-73.3+). The differences in survival and time to progression between the arms were insignificant. The median survival was 11.1 months with arm A, 9.8 months with arm B, 9.1 months with arm C and 7.5 months with arm D (P=0.62). BOLD was more toxic than DTIC.

**Conclusions:** There were no statistically significant differences in efficacy between the four treatment arms in patients with advanced melanoma but the small sample size precludes definite conclusions. However, there was a trend towards higher response rate with BOLD plus recombinant  $\alpha$  IFN-2b. The patients with soft tissue and/or lung metastases may achieve more CRs with the BOLD regimens than with the DTIC schedules whereas the patients with abdominal visceral and/or bone involvement derive little benefit from the tested regimens.

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# Hypoxia-inducible factors 1a and 2a in skin malignant melanomas

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**Background:** Hypoxia Inducible Factors 1a and 2a are a key transcription factors activating angiogenesis, glycolysis and cell migration as a response to hypoxia.

**Methods:** HIF 1a and HIF 2a cytoplasmic and nuclear expression was assessed immunohistochemically (ESEE122 and EP190b MoAbs) in a series of 46 nodular malignant melanomas of the skin treated with wide local excision. The expression of VEGF was also examined with VG1 MoAb.